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Bactericidal Activity and Pharmacology of Cefazolin

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The in vitro bactericidal activity of cefazolin was found to be similar to that of cephaloridine and cephalothin but slightly greater than that of cephalexin against a majority of 233 strains of gram-positive and gram-negative organisms. Cefazolin, however, was two- to eightfold more active than the other two drugs against *Escherichia coli* and klebsiella. The mean peak concentrations in the serum in 10 normal subjects 1 h after intramuscular injections of 1,000, 500, and 250 mg of cefazolin were 38.8, 18.6, and 12.2 μg/ml, respectively. The antibiotic could still be detected at 8 h. Peak values for a given dose of cephaloridine were comparable. However, blood levels of cefazolin were regularly higher than those of cephaloridine over the first 8 h. The mean half-life of cefazolin in the serum was 2 h, whereas that of cephaloridine was 1.4 h. The degree of serum protein-binding was strikingly higher for cefazolin (81%) than for cephaloridine (24%), suggesting that the antibacterial activity of the former in serum might be less than that of cephaloridine after equal doses. This proved to be the case when the bacterial activity of blood drawn 1, 4, and 8 h after injection of the two drugs was examined.

Cefazolin (SKF-41558-Z) is a derivative of cephalosporin C (3, 4). Its structure differs substantially from that of cephalothin, cephalexin, and cephaloridine. The antibacterial activity of cefazolin has been reported to be similar or somewhat inferior to that of the other cephalosporin derivatives against gram-positive bacteria, and moderately better against *Escherichia coli* and klebsiella (10, 12, 13).

Animal studies have indicated that cefazolin is less nephrotoxic and is excreted in bile in higher concentration than is cephaloridine (11, 12). Cefazolin undergoes no appreciable degree of biotransformation after parenteral administration (3, 6). Intramuscular injection of this agent in man results in sustained levels in the serum, and high concentrations appear in the bile (15). Protein-binding is more extensive than with any of the other cephalosporins (12).

The purposes of the present study were (i) to compare the in vitro antibacterial activity of cefazolin with that of cephaloridine, cephalexin, and cephalothin; (ii) to evaluate the effect of protein-binding on activity in vitro and in vivo; and (iii) to study the pharmacology of cefazolin and cephaloridine in normal volunteers.

MATERIALS AND METHODS

In vitro antibacterial activity. Serial twofold dilutions of each antibiotic were made by using a 96-channel automatic pipette and diluter (0.05 ml) and plastic disposable plates (Cooke Engineering Co.). Seventy strains of gram-positive and 163 strains of gram-negative bacteria were studied. An overnight growth of each organism was diluted to yield 10^6 bacteria per ml; this inoculum was dispensed in 0.05-ml volumes by using a microdel (Cooke Engineering Co.). For most organisms, the medium used for diluting the organism and antibiotic was Trypticase soy broth (Difco). Todd Hewitt broth was used for studies of *Diplococcus pneumoniae* and *Streptococcus pyogenes*, and Leventhal broth was used for *Hemophilus influenzae*.

After inoculation, the plates were incubated at 37 C; candle jars were used for *H. influenzae* and *S. pyogenes*. Eighteen hours later, the plates were examined for the presence of growth at the bottom of the well. The minimal inhibitory concentration was the maximal dilution of antibiotic in which no growth was visible. Subculture on appropriate agar was then made from clear wells, and the maximal dilution yielding no growth was considered to be the minimal bactericidal concentration.

Effect of serum. The degree of serum protein-binding of four cephalosporins was determined by an equilibrium dialysis technique (9). The initial concentration of antibiotic in either the serum or protein-free side of the chamber was 50 μg/ml.

The effect of serum on the in vitro antibacterial activity of cefazolin and cephaloridine was studied by using four gram-positive organisms. Serial dilutions

were performed as in broth studies, except that the final medium in the well consisted of 90% pooled human serum and 10% double-strength broth.

Pharmacological studies. Ten healthy young male volunteers (average age 23 years) were studied. Complete blood count, urinalysis, and examination of liver and kidney function were carried out before and after cefazolin was administered. All candidates received an intramuscular injection of 1,000 or 500 mg of cefazolin or cephaloridine; nine were given 250 mg of cefazolin as well. Venous blood was drawn 0.5, 1, 2, 4, 6, and 8 h after injection of the antibiotic. Urine was collected during the first 2 h and 2 to 8 h after treatment. All specimens were frozen (−20 C) and assayed for antibiotic concentration within 5 days by an agar diffusion method using *Bacillus subtilis* (Bacto Spore Suspension, Difco) as the test organism. Standard curves for the antimicrobial assay of specimens of urine and serum were prepared by diluting the antibiotic in saline and pooled human serum, respectively.

The "area under the curve" of antibiotic in serum was calculated by a standard method (2). The half-life of cefazolin and cephaloridine in the serum was computed by the Method of least squares (8). All statistical analyses were done by a paired *t* test.

The samples of blood drawn at 1, 4, and 8 h were also examined for antibacterial activity against four gram-positive organisms. Twofold serial dilutions of each specimen were made in pooled human serum; the inoculum, suspended in 80% pooled human serum and 20% double-strength broth, was added in a volume of .05 ml. The final medium in each well contained 90% serum and 10% double-strength broth. The dilutions ranged from 1:4 to 1:512.

RESULTS

The bactericidal activity of cefazolin for 70 strains of gram-positive bacteria was similar to that of cephaloridine and cephalothin, but slightly greater than that of cephalexin (Table 1). A similar relationship was generally noted among gram-negative bacteria, including *Hemophilus influenzae*, salmonella, and *Proteus mirabilis;* indole-positive proteus and most strains of enterobacter, serratia, and pseudomonas were resistant to 50 μg/ml of all of the cephalosporins studied (Table 2). Of interest is the finding that cefazolin appeared to be more active than the other cephalosporins and ampicillin against *E. coli* and klebsiella. The minimal bactericidal concentration for 72% of strains of *E. coli* was ≤1.6 μg of cefazolin per ml; in contrast, 6.2 to 12.5 μg/ml of cephaloridine, cephalothin, cephalexin, and ampicillin were bactericidal for this percentage of strains (Fig. 1). Cefazolin was also most active against klebsiella, although the difference was less striking than with *E. coli*. The minimal bactericidal and minimal inhibitory concentrations for both the gram-positive and gram-negative bacteria were, for the most part, within one or two dilutions.

Effect of serum. The degree of serum protein-binding of cefazolin (81%) was the highest among the four congeners examined; cephalothin, cephalexin, and cephaloridine were 68, 34, and 24% bound, respectively. The bactericidal

TABLE 1. *Minimal bactericidal activity of four cephalosporins against 70 strains of gram-positive bacteria*

Species	Antibiotic	Minimal bacterial concn (μg/ml)												Median (μg/ml)
		<.05	.05	0.1	0.2	0.4	.8	1.6	3.1	6.2	12.5	25	50	>50	
Staphylococcus aureus (penicillin sensitive), 10 strains	Cefazolin			70[a]	100										.1
	Cephaloridine		90	100											.05
	Cephalothin			10	90	100									.2
	Cephalexin								50	80	100				3.1
S. aureus (penicillin resistant), 18 strains	Cefazolin	11		39	94		100								.2
	Cephaloridine		33	61	83	100									.1
	Cephalothin	45		50	100										.2
	Cephalexin					33	44		88	100					3.1
Diplococcus pneumoniae, 20 strains	Cefazolin	95		100											<.05
	Cephaloridine	75	90	100											<.05
	Cephalothin	75		95			100								<.05
	Cephalexin	15				30	60	90	95		100				.8
Streptococcus pyogenes, 8 strains	Cefazolin			100											
	Cephaloridine		100												.05
	Cephalothin			75	88	100									.1
	Cephalexin					63		100							.4
Enterococcus, 14 strains	Cefazolin									7	28	57		100	25
	Cephaloridine					14				21	35	100			25
	Cephalothin								7		14	57		100	25
	Cephalexin											21		100	>50

[a] Cumulative percentage of strains killed by indicated concentration.

TABLE 2. *Minimal bactericidal activity of four cephalosphorins against 163 strains of gram-negative bacteria*

Species	Antibiotic	Minimal bactericidal activity (μg/ml)												Median (μg/ml)
		<.05	.05	.1	.2	.4	.8	1.6	3.1	6.2	12.5	25	50	>50	
Hemophilus influenzae, 15 strains	Cefazolin			12[a]	19	26	33	53	66	93		100			1.6
	Cephaloridine	7		14	21	28		54	80	100					1.6
	Cephalothin	7		10	26	35	42	68	94	100					1.6
	Cephalexin			19	13	26				33	53	86	93	100	12.5
Escherichia coli, 46 strains	Cefazolin					11	48	72	86	90	92	94	96	100	1.6
	Cephaloridine							28	59	76	87	91	93	100	3.1
	Cephalothin						2		6	43	74	89	93	100	12.5
	Cephalexin								2	11	74	89	93	100	12.5
Klebsiella, 26 strains	Cefazolin					19	23	54	77	89	93	97	100		1.6
	Cephaloridine							19	57	80	84		92	100	3.1
	Cephalothin							23	50	69	88		96	100	3.1
	Cephalexin							15	27	46	92	96		100	12.5
Salmonella, 9 strains	Cefazolin						11	33	89	100					3.1
	Cephaloridine							56		89		100			1.6
	Cephalothin							56		89		100			1.6
	Cephalexin							56		89		100			1.6
Enterobacter, 18 strains	Cefazolin									6	17	28	34	100	>50
	Cephaloridine										6		12	100	>50
	Cephalothin										6	17	28	100	>50
	Cephalexin													100	>50
Proteus mirabilis, 10 strains	Cefazolin								20	70	90	100			6.2
	Cephaloridine									60	100				6.2
	Cephalothin								10	60	100				6.2
	Cephalexin										40	90	100		25
P. morganii *P. rettgeri* *P. vulgaris*, 18 strains	Cefazolin												95	100	>50
	Cephaloridine													100	>50
	Cephalothin													100	>50
	Cephalexin													100	>50
Serratia, 14 strains *Pseudomonas*, 7 strains	Cefazolin													100	>50
	Cephaloridine													100	>50
	Cephalothin													100	>50
	Cephalexin													100	>50

[a] Cumulative percentage of strains killed by indicated concentration

activity of this agent against four strains of gram-positive organisms (penicillin-sensitive and resistant *Staphylococcus aureus*, *Diplococcus pneumoniae*, and *Streptococcus pyogenes*) was decreased by about 10-fold when serum was used as diluent rather than broth; in contrast, serum did not alter the activity of cephaloridine.

Pharmacological studies. Peak concentrations were achieved in the serum 1 h after the administration of cefazolin in all subjects. Mean values after doses of 1,000, 500, and 250 mg of the drug were 38.8, 18.6, and 12.3 μg/ml, respectively (Table 3). The antibiotic was still detectable in the blood after 8 h in concentrations of 4.1, 2.5 and 0.9 μg/ml. Mean values after the administration of 1,000 and 500 mg of cephaloridine were 32.7 and 19.2 μg/ml at 1 h and 1.4 and 0.5 μg/ml at 8 h, respectively. There was little variation among individual subjects (Fig. 2, 3).

The mean concentrations of cefazolin in the serum 4 to 8 h after injection were two- to fivefold higher than those of cephaloridine (Table 3). Mean levels of free antibiotic, however, calculated from the data presented in Table 3 and from the protein-binding results, were strikingly higher for cephaloridine than for cefazolin during the first 4 h (Table 4).

The percentage of drug excreted in the urine

during the first 8 h was similar for both cephalosporins (Table 3).

The "area under the curve" for cefazolin was 1.65 times greater than that for cephaloridine when both 1,000 and 500 mg were given. The difference between the two agents was highly significant ($P < 0.001$) by a paired t test. When these values were corrected for serum protein-binding, however, the "area under the curve" of free cephaloridine 2.45 times greater than that of cefazolin at both dose levels.

Half-life of cefazolin. The mean and standard errors of the half-life of cefazolin were 2.02 ± 0.13 and 2.02 ± 0.11 h with the 1,000- and 500-mg doses, respectively. Comparable values for cephaloridine were 1.37 ± 0.11 and 1.08 ± 0.08 h. The half-life of cefazolin was significantly longer than that of cephaloridine at both doses ($P < 0.01$).

Antibacterial activity in research subjects. The antibacterial activity of the serum after intramuscular injection of 500 mg of cefazolin or cephaloridine against penicillin-sensitive *Staphylococcus aureus* is shown in Fig. 4. Bactericidal titers of cephaloridine were strikingly higher than those of cefazolin for both penicillin-sensitive and resistant strains of *S. aureus* at all intervals of time studied. Only one treated subject exhibited bactericidal activity at a dilution of 1:4 against the penicillin-sensitive organism at 8 h. In contrast, the sera of all recipients of cephloridine were bactericidal at a dilution of 1:8 or higher at this time (Fig. 4). Both *D. pneumoniae* and *S. pyogenes* were killed at serum dilutions of 1:32, 1:8, and 1:4 at 1, 4, and 8 h after the injection of 500 mg of cefazolin.

Pain tolerance. The degree of pain associated with the intramuscular injection of 500 or 1,000 mg of either cefazolin or cephaloridine dissolved in no less than 2 or 3 ml of water was compared, in double blind fashion, in nine subjects. Cefazolin appeared to be more painful in only one of these individuals; in the others, there was no difference in the degree of discomfort. This observation is in agreement with the results of another study (6).

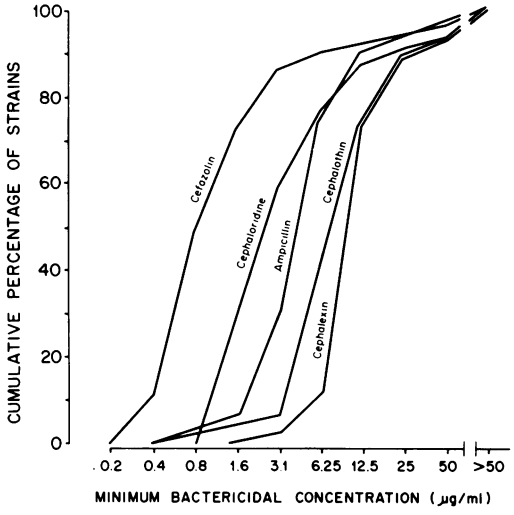

FIG. 1. *Bacteridical activity of four cephalosporins and ampicillin against 46 strains of Escherichia coli.*

DISCUSSION

The in vitro data presented here indicate that the antibacterial activity of cefazolin against gram-positive and gram-negative organisms is similar to that of cephlothin and cephaloridine and somewhat greater than that of cephalexin. Cefazolin appears to be considerably more potent than the other cephalosporins or ampicillin against *E. coli;* this confirms previously published data (10, 15). There is also an appreciable bactericidal effect against enterococci; this is in contrast to the observations of others (5, 14).

The mean peak concentrations of cefazolin in serum were noted to be similar to those of cephaloridine after the administration of equal doses (14). In contrast, the results of several other studies indicate markedly higher serum levels of cefazolin than of cephaloridine after exhibition of the same quantity of each drug (1, 12). The explanation for the discrepancy is not clear, since serum concentrations of drugs rep-

TABLE 3. *Mean serum concentration and urinary excretion of cefazolin and cephaloridine in 10 normal subjects*

Antibiotic	Intramuscular dose (mg)	Mean serum concn (μg/ml)						Urinary excretion (%)		Total
		0.5 h	1 h	2 h	4 h	6 h	8 h	0–2 h	2–8 h	0–8 h
Cefazolin	1,000	34.4	38.8	34.0	17.9	9.0	4.1	28	46	74
Cephaloridine	1,000	26.9	32.7	21.7	8.8	3.0	1.42	46	41	87
Cefazolin	500	17.1	18.6	16.8	8.8	4.7	2.5	26	40	66
Cephaloridine	500	18.2	19.2	11.0	2.9	1.1	.5	26	26	52
Cefazolin	250	11.8	12.3	9.6	4.4	1.4	.9	21	51	72

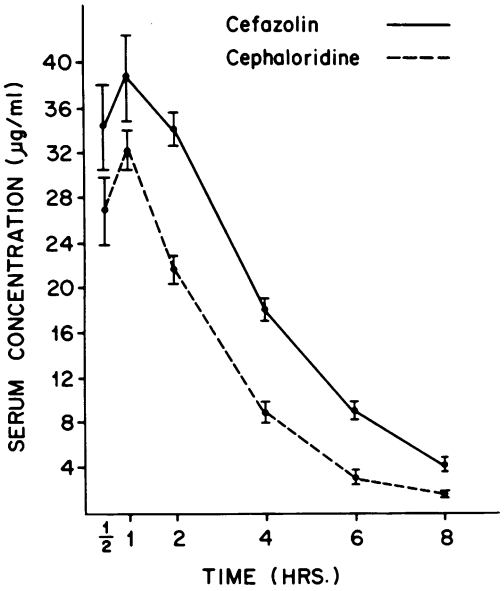

FIG. 2. *Mean concentration of antibiotic in the serum of 10 subjects after intramuscular injection of 1,000 mg of cefazolin or cephaloridine. The vertical bars represent standard error of the mean.*

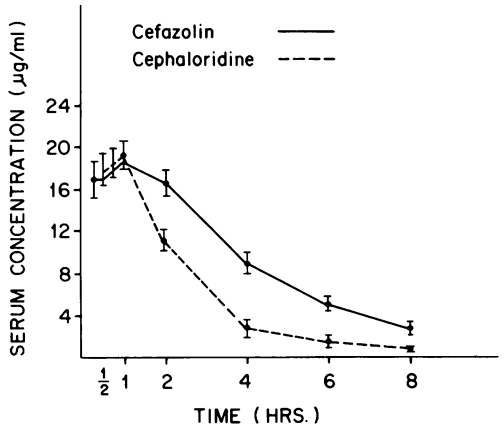

FIG. 3. *Mean concentration of antibiotic in the serum of 10 subjects after intramuscular injection of 500 mg of cefazolin or cephaloridine. The vertical bars represent standard error of the mean.*

resent the results of many factors (7). The half-life of cefazolin in serum was considerably longer than that of cephaloridine after intramuscular injections; thus, the "area under the curve" of the former was 1.6 times greater than that of the latter.

The degree of serum protein-binding of cefazolin (81%) was markedly higher than that of cephaloridine (24%). This is probably the reason for the striking reduction in the in vitro activity of cefazolin when serum, rather than broth, was used as diluent. Since only free drug appears to be bactericidal, the degree of protein-binding must be taken into account when circulating levels of antibiotics are compared (7). In addition, studies in our laboratory suggest that the quantity of an antibiotic in subcutaneous fibrin loci is directly related to the peak concentrations of free antibiotic in the serum (unpublished data). The present studies indicated that maximal levels of free cephaloridine were several times higher than those of cefazolin. However, this does not necessarily imply that cephaloridine is preferable to cefazolin in clinical situations since concentrations in specific organs, such as liver and kidney, may exceed those in the blood. Furthermore, differences in the degree of nephrotoxicity (12) and in activity against specific pathogens may confer a significant advantage to cefazolin.

TABLE 4. *Mean concentration of free cefazolin and cephaloridine in serum of 10 normal subjects*

Antibiotics	Intramuscular dose (mg)	Mean concn of free antibiotic in serum (μg/ml)[a]					
		0.5 h	1 h	2 h	4 h	6 h	8 h
Cefazolin	1,000	6.5	7.3	6.4	3.4	1.7	.8
Cephaloridine	1,000	20.4	24.8	16.5	6.7	2.3	1.1
Cefazolin	500	3.2	3.5	3.2	1.7	0.9	0.5
Cephaloridine	500	13.8	14.6	8.3	2.2	0.8	0.4
Cefazolin	250	2.2	2.3	1.8	0.8	0.3	0.2

[a] Calculation from total serum concentration and protein-binding studies.

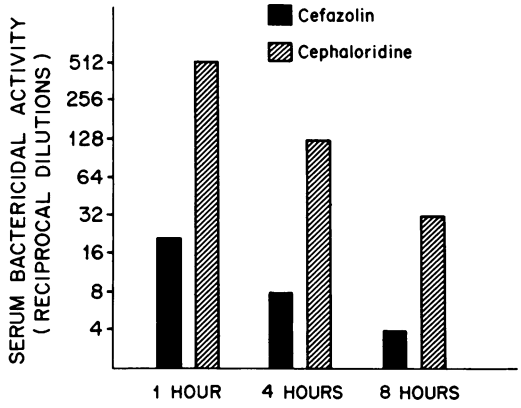

FIG. 4. *Mean serum bactericidal activity against a penicillin-sensitive Staphylococcus aureus in 10 subjects after intramuscular injection of 500 mg of cefazolin or cephaloridine.*

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